EVALUATION OF *INVITRO* ANTI CANCER ACTIVITY OF ETHANOL AND AQUEOUS *EXTRACTS OF CAYRATIA CARNOSA* GAGNEP

Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY,

CHENNAI - 32.

In partial fulfillment for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

Submitted by

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OCT -2017

CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"Evaluation of in vitro anticancer activity of ethanolicand aqueous extract of *Cayratia Carnosa Gagnep"*** submitted to "The Tamilnadu Dr.M.G.R. Medical University", Chennai. in partial fulfillment to the requirement for the award of Degree of **Master of Pharmacy in Pharmacology**,is a bonafide work carried out by **Mr.M.K.SAIFUDHEEN, Reg.No:261525208**,during the academic year 2016-2017, under my guidance and direct supervision in the department of pharmacology, J.K.K.Nataraja College of Pharmacy, Komarapalayam.

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DECLARATON

I hereby declare that the dissertation **"Evaluation of in vitro anticancer activity of ethanolic and aqueous extract of *Cayratia Carnosa Gagnep''***, has been carried out under the guidance and supervision of Mr.V.Vvenkateswaran, M.Pharm, Assistant Professor, Department of Pharmacology, J.K.K. Nataraja College of Pharmacy. Komarapalayam, in partial fulfillment of the requirements for the award of degree of Master of Pharmacy in Pharmacology during the academic year 2016-2017

I further declare that, this work is original and this dissertation has not been submitted previously for the award of any other degree,diploma associate ship and fellowship or any other similar title.

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Dedicated to

Parents,

Teachers&

My Family

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Mr.M.K.SAIFUDHEEN,

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CONTENTS

1. INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, well into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. Indeed, about 25% of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound.[1]

Undisputedly, the history of herbology is inextricably intertwined with that of modern medicine. Many drugs listed as conventional medications were originally derived from plants. Salicylic acid, a precursor of aspirin, was originally derived from white willow bark and the meadowsweet plant. Cinchona bark is the source of malaria-fighting Quinine. Vincristine, used to treat certain types of cancer, comes from Periwinkle. The Opium poppy yields morphine, codeine, and paregoric, a treatment for diarrohea Laudanum, a tincture of the Opium poppy, was the favored tranquilizer in Victorian times. Even today, Morphine-the most important alkaloid of the Opium poppy-remains the standard against which new synthetic pain relieves is measured.

Prior to the discovery and subsequent synthesis of antibiotics, the herb Echinacea (which comes from the plant commonly known as purple coneflower) was one of the most widely prescribed medicines in the United States. For centuries, herbalists prescribed Echinacea to fight infection. Today, research confirms that the herb boosts the immune system by stimulating the production of disease-fighting white blood cells.[2, 3]

The use of plants as medicine is older than recorded history. As mute witness to this fact marshmallow root, hyacinth, and yarrow have been found carefully tucked around the bones of a Stone Age man in Iraq. These three medicinal herbs continue to be used today. Marshmallow root is a demulcent herb, soothing to inflamed or irritated mucous membranes, such as a sore

throat or irritated digestive tract. Hyacinth is a diuretic that encourages tissues to give up excess water. Yarrow is a time-honored cold and fever remedy that may once have been used much as aspirin is today.

The entire Middle East has a rich history of herbal healing. There are texts surviving from the ancient cultures of Mesopotamia, Egypt, and India that describe and illustrate the use of many medicinal plant products, including castor oil, linseed oil, and white poppies. In the scriptural book of Ezekiel, which dates from the sixth century B.C., we find this admonition regarding plant life: " the fruit thereof shall be for meat, and leaf thereof for medicine". Egyptian hieroglyphs show physicians of the first and second centuries A.D. treating constipation with Senna pods, and using Caraway and Peppermint to relieve digestive upsets.[4]

Herbal Medicine Today

The World Health Organization (WHO) estimates that about 80% of the world population presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous people's traditional medicine and a common element in Ayurvedic, Homeopathic, Naturopathic, traditional oriental, and native American Indian medicine. WHO notes that of 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value.

Rather than using a whole plant, pharmacologists identify, isolate, extract, and synthesize individual components, thus capturing the active properties. This can create problems, however. In addition to active ingredients, plants contain minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavanoids and other substances that are important in supporting a particular herb's medicinal properties. These elements also provide an important natural safeguard. Isolated or synthesized active compounds can become toxic in relatively small doses. It usually takes a much greater amount of a whole herb, with all of its components, to reach a toxic level. Herbs *are*medicines, however, and they can have powerful effects. They should not be taken lightly. The suggestions for herbal treatments in this book are not intended to substitute for consultation

with a qualified health care practitioner, but rather to support and assist you in understanding and working with your physician's advice.[5]

Substances derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma, and other problems. For example, Ephedra is an herb used in Traditional Chinese Medicine for more than two thousand years to treat asthma and other respiratory problems. Ephedrine, the active ingredient in Ephedra, is used in the commercial pharmaceutical preparations for the relief of asthma symptoms and other respiratory problems. It helps the patient to breathe more easily.

Another example of the use of an herbal preparation in modern medicine is the Foxglove plant. This herb had been in use since 1775. At present, the powdered leaf of this plant is known as the cardiac stimulant digitalis to the millions of heart patients it keeps alive worldwide.

There are over 750,000 plants on earth. Relatively speaking, only a very few of the healing herbs have been studied scientifically. And because modern pharmacology looks for one active ingredient and seeks to isolate it to the exclusion of all the others, most of the research that is done on plants continues to focus on identifying and isolating active ingredients, rather than studying the medicinal properties of whole plants. Herbalists, however, consider that the power of a plant lies in the interaction of all its ingredients. Plants used as medicines offer synergistic interactions between ingredients both known and unknown.

The efficacy of many medicinal plants has been validated by scientist’s abroad, from Europe to the orient. Thanks to modern technology, science can now identify some of the specific properties and interactions of botanical constituents. With this scientific documentation, we now know why certain herbs are effective against certain conditions. However, almost all of the current research validating herbal medicine has been done in Germany, Japan, China, Taiwan, and Russia. And for the most part, the United States Food and Drug Administration (FDA), which is responsible for licensing all new drugs (or any substances for which medicinal properties are claimed) for use in the United States, does not recognize or accept findings from across the sea. Doctors and government agencies want to see American scientific studies before recognizing the effectiveness of a plant as medicine. Yet even though substantial research is being done in other countries, drug companies and laboratories in the United States so far have

not chosen to put much money or resources into botanical research. The result is that herbal medicine does not have the same place of importance or level of acceptance in this country as it does in other countries. [6]

Alternative System of Medicine

Failure to control stress-related environmental and psychological illness, history of surgical accidents and growing resistance to antibiotics, people are looking for alternative medicines. Moreover, there are some chronic illnesses such as asthma, arthritis, irritable bowl syndrome, migraine, insomnia, immunodeficiency, cancer, jaundice, which do not have effective conventional treatment. Majority of the patient with cancer or acquired immunodeficiency syndrome are currently using one or more alternative therapies. Negative side effects of conventional treatment are also encouraging people to look for alternative system of medicine. Above all, after the FDA's decision to categorize herbal remedies as food supplements, the popularity of herbal products is increasing day by day. There is no restriction and one can buy over the counters. Advertising and promotion of these products cannot separate myths from reality and give a ray of hope to some patients. Hence, estimates of herbal medicine sales skyrocketed to between $2 and $3 billion.

It has been estimated that 70-90% of the world's population relies on alternative therapies and practices. Moreover, many types of alternative therapies are a formal approach to healthcare in various societies and culture around the world. If a particular therapeutic approach has not originated by American health care it does not render it worthless, quackery or fad. Most alternative therapies have evolved from ancient healing system in various cultures around the world and are based on reasonable scientific background. There must be some truth in it. As science catches up with human behavior some types of alternative therapies are being found useful.[7]

Demand of Herbal Medicine

Despite the dramatic advancement and advantages of conventional medicines herbal drug have much to offer? Today, herbal drugs are coming back into prominence. Side effects of the conventional medicines such as antibiotics, antimicrobial agents are the major problems. Over

the years, some of the infectious organisms have developed resistance to synthetic drugs too. Medicinal chemist is taking for more potent and effective drugs hence more complications.

Herbals are used in the art of healing since the time immemorial. The primitive man through trial and error gained knowledge of herbal and passed it on to the next progeny. It is reasonable to assume that for ten thousands of year herbs were perhaps used for the magical power as well as for their medicinal values.

Despite the development in modern medicines, the use of herbs is still increasing, why? For thousands of year herbs and other products from the natural source have been used in treating various diseases. Some of those in current use have been an ancient heritage, whereas other has arisen from discoveries and cultural trend in more recent centuries. All therapy referred to as alternative or complementary came from outside the main stream.[8,9]

Role of medicinal plants in anticancer treatment

Cancer is the abnormal growth of cells in our bodies that can lead to death. Cancer cells usually invade and destroy normal cells. These cells are born due to imbalance in the body and by correcting this imbalance, the cancer may be treated. Billions of dollars have been spent on cancer research and yet we do not understand exactly what cancer is. Every year, millions of people are diagnosed with cancer, leading to death. According to the American Cancer Society, deaths arising from cancer constitute 2–3% of the annual deaths recorded worldwide. Thus cancer kills about 3500 million people annually all over the world. Several chemopreventive agents are used to treat cancer, but they cause toxicity that prevents their usage[10].

In India, there are atleast 250,000 species of plants out of which more than one thousand plants have been found to possess significant anticancer properties.Cancer is often deadly and affects a considerable number of people worldwide. Ongoing research is being done throughout the world to seek out effective treatments for cancer, including the use of plants to relieve and treat cancer patients. This treatment makes use of the compounds naturally present in plants that are known to inhibit or kill carcinogenic cells. An alternative to chemotherapy, which is the most common means by which doctors and specialists treat cancer, organically based treatments may not have the severe side effects that radial treatments and chemotherapy has. The harsh side

effects of cancer treatments is one motivating factor to finding alternative methods. The use of botanical when treating cancer patients is considered a natural alternative, because some plants may contain properties that naturally have the ability to prevent the spread or risk of developing various forms of cancer. As in all medical testing, careful precautions and considerations are taken when studying the different compounds present in plants that are known to treat cancer.

Some examples of plants that may be used for cancer treatment are discussed below with their respective advancements. There are around 460 species of plants that can be used as herb for remedy, including plant healer various types of cancer.Various types of anti-cancer plant are Zedoary (*Curcumazedoaria*), Rodent Tuber(*Typhoniumflagelliforme*), God's Crown (*Phaleriamacrocarpa*), Madagaskar Periwinkle (*Catharanthusroseus*), Artocarpus Integer (*Selaginellacorymbosa*), Bamboo Grass *LoathatreumGràcies*), handsome (*Taraxacummongolicum*), fruit makasar (*Bruccajavanica*), Garlic (*Alliumsativum*), Echo China (*Smilaxchina*), Sunflower (*Helianthusannus*), Leunca (*Solanumnigrum*), Job's Tears (*CoixLachryma-Jobi*), Bamboo Rope (*Asparaguscochinchinensis*), and others[11].

TUMOR[12]:

Neoplasia literally means the process of "new growth," and a new growth is called a neoplasm. The term tumor was originally applied to the swelling caused by inflammation. Neoplasms also may induce swellings, but by long precedent, the non-neoplastic usage of tumor has passed into limbo; thus, the term is now equated with neoplasm. Oncology (Greek oncos = tumor) is the study of tumors or neoplasms. Cancer is the common term for all malignant tumors. Although the ancient origins of this term are somewhat uncertain, it probably derives from the Latin for crab, cancer-presumably because a cancer "adheres to any part that it seizes upon in an obstinate manner like the crab."

1. DEFINITIONS:

Solid tumors are defined as abnormal masses of tissue that usually do not contain cysts or liquid areas. Solid tumors may be benign (not cancerous), or malignant (cancerous). A number of malignant diseases are often also categorized as “solid tumors” such as breast cancer, cancer of the pancreas, lung, colon, etc.

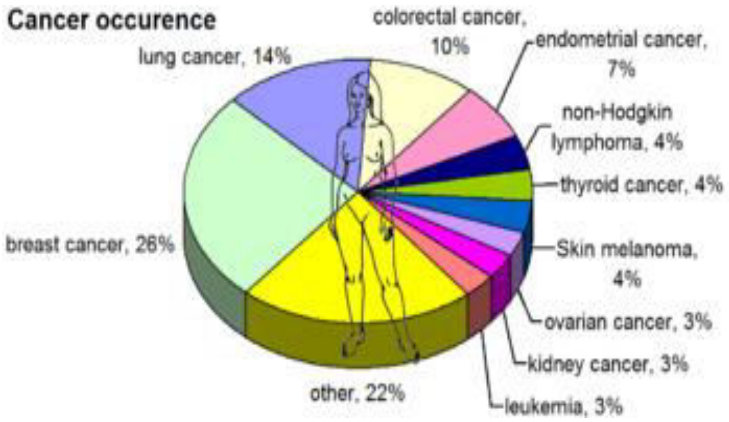
Solid tumors can be split into three separate categories, depending on the type of cells from which they typically arise in the patient’s body, which include:

- **Sarcomas:** Cancers arising from connective or supporting tissues such as bone or muscle.
- **Carcinomas:** Cancers arising from the body's glandular cells and epithelial cells, which line the air passages and gastrointestinal tract.
- **Lymphomas:** Cancers of the lymphoid organs such as lymph nodes, spleen, and thymus, which produce and store infection-fighting cells. Lymphoma is cancer of the lymphatic system, which is part of the immune system.

Definition

Breast cancer is caused by the development of malignant cells in the breast. The malignant cells originate in the lining of themilk glands or ducts of the breast (ductal epithelium), defining this m alignancy as a cancer. Cancer cells are characterized byuncontrolled division leading to abnorma

l growth and the ability of these cells to invade normal tissue locally or to spreadthroughout the b ody, in a process called metastasis.



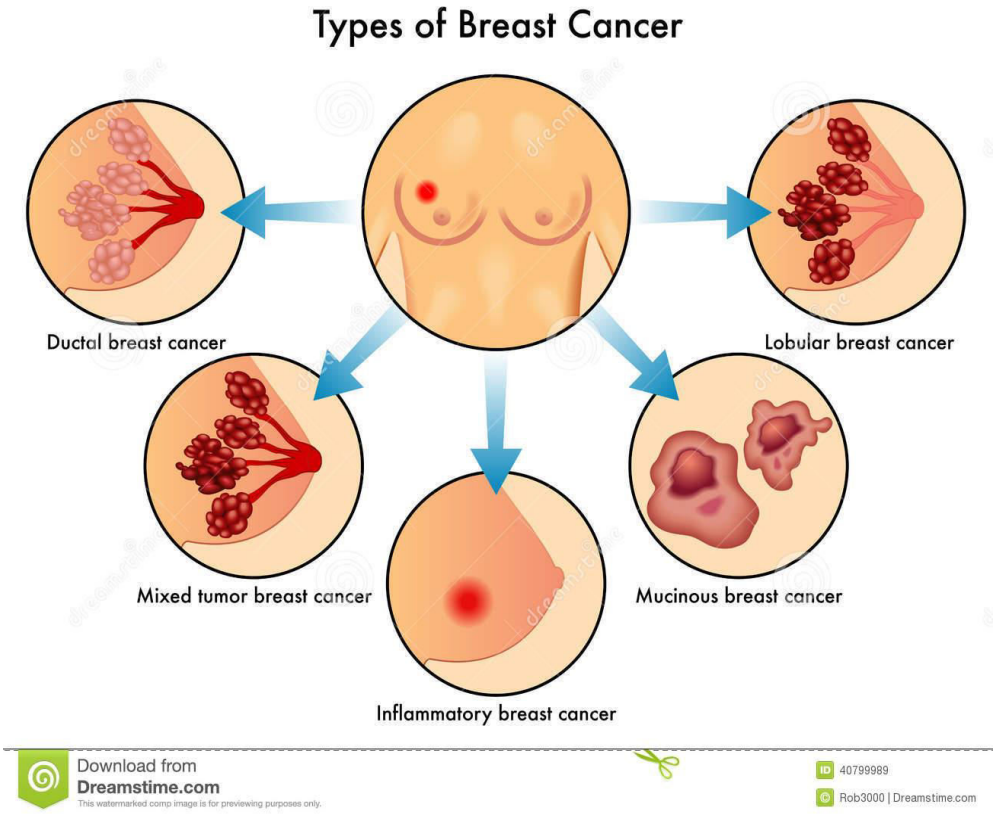
Description

Breast cancer arises in the milkproducing glands of the breast tissue. Groups of glands in normal breast tissue are calledlobules. The products of these glands are secreted into a duct system that l eads to the nipple. Depending on where in theglandular or ductal unit of the breast the cancer aris es, it will develop certain characteristics that are used to subclassifybreast cancer into types. The pathologist will note the subtype at the time of evaluation with the microscope. Ductalcarcinoma begins in the ducts, lobular carcinoma has a pattern involving the lobules or glands. The more im portantclassification is related to the evaluated tumor's capability to invade, as this characteristic defines the disease as a truecancer. The stage before invasive cancer is called *in situ*, meaning tha t the early malignancy has not yet become capable ofinvasion. Thus, ductal carcinoma in situ is c onsidered a minimal breast cancer.The International Agency for Research on Cancer estimates of the incidence of mortality and prevalence from major types of cancer, at national level, for 184 countries of the world revealed that there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide [13].By 2030, it is projected that there will be 26 million new cancer cases and 17 million cancer deaths per year [14]

How breast cancer spreads?

The primary tumor begins in the breast itself, but once it becomes invasive, it may progress beyo nd the breast to the regionallymph nodes or travel (metastasize) to other organ systems in the bod y and become systemic in nature. Lymph is the clear,proteinrich fluid that bathes the cells throug hout the body. Lymph will work its way back to the bloodstream via smallchannels known as ly mphatics. Along the way, the lymph is filtered through cellular stations known as nodes, thus the y arecalled lymph nodes. Nearly all organs in the body have a primary lymph node group filterin g fluid that comes from that organ.In the breast, the primary lymph nodes are under the armpit, o r axilla. Classically, the primary tumor begins in the breast andthe first place to which it is likely to spread is the regional lymph nodes. Cancer, as it invades in its place of origin, may alsowork it s way into blood vessels. If cancer gets into the blood vessels, the blood vessels provide yet anot her route for thecancer to spread to other organs of the body.Breast cancer follows this classic pr ogression though it often becomes systemic or widespread early in the course of thedisease. By t he time one can feel a lump in the breast it is often 0.4 inches, or one centimeter, in size and cont ains roughly amillion cells. It is estimated that a tumor of this size may take one to five years to d evelop. During that time, the cancer maymetastasize, or spread by lymphatics or blood to areas el sewhere in the body.

When primary breast cancer spreads, it may first go to the axillary nodes. If this occurs, regional metastasis exists. If itproceeds elsewhere either by lymphatic or bloodborne spread, the patient d evelops systemic metastasis that may involve anumber of other organs in the body. Favorite sites of systemic involvement for breast cancer are the lung, bones, liver, skin,and soft tissue. As it tu rns out, the presence of, and the actual number of, regional lymph nodes containing cancer remai nsthe single best indicator of whether or not the cancer has become widely metastatic. Because te sts to discover metastasis inother organs may not be sensitive enough to reveal minute deposits o f cancer cells, the evaluation of the axilla for regionalmetastasis becomes very important in maki ng treatment decisions for this disease.If breast cancer spreads to other major organs of the body, its presence will compromise the function of those organs. Deathis the result of extreme compro mise of vital organ function.



TYPES OF BREAST CANCER.

- Ductal carcinoma in situ: The most common type of noninvasive breast cancer is ductal carcinoma in situ (DCIS). This type of cancer has not spread and therefore usually has a very high cure rate.
- Invasive ductal carcinoma: This cancer starts in a duct of the breast and grows into the surrounding tissue. It is the most common form of breast cancer. About 80% of invasive breast cancers are invasive ductal carcinoma.
- Invasive lobular carcinoma: This breast cancer starts in the glands of the breast that produce milk. Approximately 10% of invasive breast cancers are invasive lobular carcinoma.

- The remainder of breast cancers are much less common and include the following:
- Mucinous carcinoma are formed from mucus-producing cancer cells. Mixed tumors contain a variety of cell types.
- Medullary carcinoma is an infiltrating breast cancer that presents with well-defined boundaries between the cancerous and noncancerous tissue.
- Inflammatory breast cancer: This cancer makes the skin of the breast appear red and feel warm (giving it the appearance of an infection). These changes are due to the blockage of lymph vessels by cancer cells.
- Triple-negative breast cancers: This is a subtype of invasive cancer with cells that lack estrogen and progesterone receptors and have no excess of a specific protein (HER2) on their surface. It tends to appear more often in younger women and African-American women.
- Paget's disease of the nipple: This cancer starts in the ducts of the breast and spreads to the nipple and the area surrounding the nipple. It usually presents with crusting and redness around the nipple.
- Adenoid cystic carcinoma: These cancers have both glandular and cystic features. They tend not to spread aggressively and have a good prognosis.
- Lobular carcinoma in situ: This is not a cancer but an area of abnormal cell growth that can lead to invasive breast cancer later in life.

RISK FACTORS[13-14]

- ✓ Modifiable risk factor(thing that people can change themselves such as consumption of alcoholic beverages).
- ✓ Fixed risk factors ((thing that people cannot changed, such as age and biological sex).
- ✓ Smoking increase the risk of breast cancer.
- ✓ A number of dietary factors have been linked to the risk of breastcancer.

Symptoms of cancer

The symptoms and signs of lymphoma are very similar to those of simple illnesses such as viral illnesses and the common cold, and this can cause problems with delayed diagnosis.

The difference is that the symptoms of lymphoma persist long after the usual run of a viral infection.

- Weight loss and loss of appetite
- Unusual itching
- Fatigue
- Pain or altered sensation
- Loss of appetite
- Breathlessness
- Enlarged tonsils

Treatments and prevention[15]

A number of treatment options are used against lymphoma cancer these are

- Radiation therapy
- Biologic therapy
- Antibody therapy
- Stem-cell transplantation
- Steroid treatment

CANCER CELL LINES AS A MODEL FOR CANCER STUDY[15]

Cancer cell lines have been widely used for research purposes and proved to be a useful tool in the genetic approach, and its characterization shows that they are, in fact, an excellent model for the study of the biological mechanisms involved in cancer. The use of cancer cell lines allowed an increment of the information about the deregulated genes and signalling pathways in this disease . Furthermore, the use of the cell model was in the origin of the development and testing of anticancer drugs presently used and in the development of new therapies , but also as an alternative to transplantable animal tumours in chemotherapeutics testing . In fact, the use of the appropriate *in vitro* model in cancer research is crucial for the investigation of genetic, epigenetic and cellular pathways for the study of proliferation deregulation, apoptosis and cancer progression , to define potential molecular markers and for the screening and characterization of cancer therapeutics. The results of the research in cancer cell lines are usually extrapolated to *in vivo* human tumours and its importance as models for drug testing and translational study have been recognized by many biomedical and pharmaceutical companies.

Cancer cell line	**Species**		**Morphology**
HeLa	Homo sapiens	Cervix adenocarcinoma	Epithelial
MCF-7	Homo sapiens	Breast adenocarcinoma	Epithelial
U87MG	Homo sapiens	Glioblastoma-astrocytoma	Epithelial
HT-29	Homo sapiens	Colon adenocarcinoma	Epithelial
A549	Homo sapiens	Lung carcinoma	Epithelial
HEP-G2	Homo sapiens	Hepatocellular carcinoma	Epithelial
K-562	Homo sapiens	Chronic myeloid leukaemia	Lymphoblas
Cos7	Homo sapiens	SV40 transformed - kidney	Fibrobla

2. LITERATURE REVIEW

History of Medicine goes back practically to the existence of human being. The currentaccepted Modern Medicine or Allopathy has gradually been developed over the years by thescientific observational efforts of scientists. However, the basis of its development remains in theroots of traditional medicine and therapies. The history of medicine includes many ludicroustherapies. Nevertheless, the ancient wisdom has been the basis of modern medicine and willremain as one of the important source of future medicine and therapeutics. The future will be more holistic, personal and involve wise-use of ancient and modern therapeutic skills in acomplementary manner so that maximum benefits will go to the patients and the community.

Medical system has a broad meaning. It incorporates diagnosis, prognosis and prevention.Treatment has a definite rational and philosophy. Therapies have a limited meaning referring tomainly treatment. Within this framework of definition, two prominent systems of medicine withhistory of existence for thousands of years include Indian system of medicine such as Siddha,Ayurveda, etc. and Chinese Medicine. Ayurveda incorporates Yoga while Chinese medicineincludesAcupuncture.Systematic literature survey is the main basis for the planning of any scientific work and due to this the review of literature regarding the *cayratiacarnosa (wall .ex. wight)* gengap.has been examined and reported. They are in following manner pharmacogonostical, phytochemical and pharmacological review.

Shah *et al*. (1970) has done micro-histogenic studies on tendrils on vitaeace[16].

Duthie*et al*.(1980) has reported presence of delphinidin and cyanidin in the leaves of *cayratiacarnosa (wall.ex. wight)* gengap[17].

Singh *et al*. (1982) has reported yellow waxy oil and sterols in the leaves of *Cayratiacarnosa (wall.ex. wight)* gengap[17].

Patil*et al*. (2000) has been done the phytochemical studies and found the presence of lignins , tannins, raphides, mucilaginous substances and flavonoids in *CayratiaCarnosa (wall.ex. wight)* gengap[18.]

Perumal*et al*. (2014) identified a novel PPARγ agonist from GC-MS analysis of ethanolic extract of *Cayratia trifolia*[19].

Perumal*et al.* (2015) performed the Isolation, structural characterization and in silico drug-like properties prediction of bioactive compound from ethanolic extract of *Cayratia trifolia*[20].

Pharmacological review:

Kundu*et al.* (2000) investigated the Antitumor activity of epifriedelanol from Vitistrifolia. Fitoterapia[21].

Gupta *et al.* (2012)Evaluated the gastric anti-ulcer activity of methanolic extract of *Cayratiatrifolia* in experimental animals[22].

Perumal*et al.* (2012).Studied the *Invitro* antioxidant activities and HPTLC analysis of ethanolic extract of *Cayratia trifolia*[23].

Sowmya*et al.* (2015) investigated the In vitro Antioxidant Activity, In vivo Skin Irritation Studies and HPTLC Analysis on extracts of Cayratiatrifolia[24].

Palanisamy*et al.* (2016) studied theAssessment of dual inhibitory activity of epifriedelanol isolated from *Cayratiatrifolia* against ovarian cancer. They reported the properties of the epifriedelanol and scope to develop the compound as a potent anti-ovarian cancer drug[25.]

Miscellaneous review:

Patil*et al*. (2005) has been done preliminary evaluation of indigenous wild grapegermplasm for infestation of caterpillar[26].

Herbs with cytotoxic studies

Ahmed *et al*. (2003) reportedthe chemotherapeutic and chemoprotective effects of extract of *Nigella sativa* which is containing anticancer principles of quinones that include thymoquinone (TQ) and dithymoquinone[27].

Jain *et al*. (2011) evaluated the cytotoxic effect of five alcoholic plant extracts on MCF-7 and HL-60 cells using MTT assay. MTT Different dilutions of extracts were treated and IC_{50} values

were calculated. From their study, they concluded that the *S. grandiflora*bark extract has potent *in vitro*cytotoxic activity against cancer cell lines[28].

Punitha*et al*. (2012) studied the cytotoxicity of ethanolic leaf extracts of *Gmelinaarborea*(Verbenaceae) was tested against Colon cancer (COLO 201), Gastric cancer (HT-29) and Human oesophagel cancer (TE-2) cell lines using the thiazolyl blue test (MTT) assay[29].

Rani *et al*. (2013) evaluated the anticancer property of the elected four seaweeds viz., *Sargasssumwightii*Greville, *Padinatetrastromatica*Hauck, *Ulvafaciata*Delile and *Gracillariaedulis*and*P.C. Silva*from the south east coast of India. The cytotoxic activity was determined against human A-549 lung adenocarcinoma cancer cell lines and VERO (African green monkey kidney cells) non-cancerous cell line using the MTT method[30].

Sumathi*et al.* (2013) performed thecomparative study of the apoptosis influencing activity of methanolic extract of *Prosopis cineraria* leaves in breast cancer cell line MCF-7[31].

3. PLANT PROFILE [32-35]

BOTANICAL NAME: *Cayratiatrifolia*(wall .ex. wigh&Arn) gagnep.

Synonym : *Cayratiacarnoca*gagnep.

*Cissusauriculata*Roxb.

*Vitis*carnosa

*Vitis*trifolia

Taxonomy

- Kingdom : Plantae
- Subkingdom : Viridaeplantae
- Phylum : TracheophytaSinnott,
- Subphylum : Spermatophytina (auct.)
- Infraphylum : Angiospermaeauct.
- Class : MagnoliopsidaBrongniart
- Subclass : Rosidae
- Superorder : Vitanae
- Order : Vitales
- Family : Vitaceae
- Genus : Cayratia
- Species : CarnosaGagnepain

Description

A somewhat fleshy climbing shrub,usually pubescent when young,tendrils short, slender and branched,leaves trifoliate, usually pubescent, lecillets dentate, flowers in branched, long pedunculedcymes, flower buds globose,fruits fleshy berries,seeds pyriform or triangular , rounded and rugose on the back

Habitat

Throughout India on the hills, west coast and western charts in India.This species are globally distributed in Indo-Malesia. Within India, it is found in the hotter parts from Jammu and Rajasthan, Assam, Tripura and West Bengal, extending into Peninsular India, up to an altitude of 600 m.

Part Used

Whole plant

Chemical constituents

Hydrocyanic acid, delphinidin and cyanidine

BOTANTICAL CHARACTERS

Stem

Woody, compressed when young; tendrils short, slenderusuallybranched.

Leaves

Leaves are 3-foliolate,common petioles,2- 4.5cm. leaflets thick, 3.8-5.7 by 2.2-3.2cm. Ovate- lanceolate, more or less pubescent on both surface; main nerve 5-6 pairs; petioles of the lateral leaflets 3-6mn; stipules small, ovate acute

Flower

Flowers in branched divaricate pubesecent long- peduncled cymes; buds globose; pedicals about 3 mm. long, calyx pubesent outside, funnel shaped, 4- lobed. Petals 4oblong.

Seed

Triangular, rounded and rugose on the back, cuneate on the face.

Ethanomedicinal uses

- The root has sharp sour taste used for “vata” and “Kapha”.
- The plant is used for the treatment of tumors, pains and spleen complaints **(Ayurveda).**
- The plant is used for purification of blood **(Yunani).**
- The plant is used for dropsy, wounds, ulcer and cardiac disorder.
- It has been also used in the treatment of yoke sores on the necks of bullocks for that purpose, a poultice of the leaves is employed**.**
- Leaves are used in embrocating**.**
- Fermentations with hot decoction is recommended in fever.

4. AIM AND SCOPE OF STUDY

As we know very well that everything in this world change time by time, since thousands of years the era was of Ayurveda or herbal origin drug. But last few decades, it was replaced by allopathic system of medicine, which was accepted worldwide but later due to its lots of adverse effect again men step down on Ayurveda because of its better therapeutic result and safety profile and now the people are more believing in natural origin drugs. But prior clinical trial; of medicinal drug preparation and proper pharmacological and toxicological studies are required to ensure the safety of the drug.

The present work focused the comprehensive study of*cayratiacarnosa (wall.ex.wight)* gengap. The ethnobotanical review showed that the entire plant has potent medicinal properties such as wound healing, analgesic, anti-inflammatory, antiulcer and antitumor properties. Till now, few phytochemical studies has been done on this plant. Therefore, this plant having wide scope for detail pharmacogonostical, preliminary phytochemical and pharmacological investigation.

5. PLAN OF WORK

The plan of work on the present studyincludes the following.

1. Priliminary Phytochemical studies
    a. Preparation of extracts
    b. Qualitative phytohemical analysis
2. Pharmacological studies
    a. *Invitro*anti cancerstudies

6. MATERIALS AND METHODS

6.1 PROCEDURE:

Fresh mixture were**collected** and was dried. After drying they were again pulverized. The size is reduced. The dried plant *Cayratiatrifolia*Gagnep, powder mixture was weighed about 250g Extracted by soxhlet apparatus using 99% of ethanol and water as a solvent for 72 hours. The yield of product was 7.358g.

Collection of Specimen

The species for the proposed study that is *cayratiacarnosa (wall .ex. wight&Arn.)*Gagnep.familyVitaceae was collected from surroundings of vattamalai, Komarapalayam. Further it was identified and authenticated by **Dr. P. Jayaraman,** Botanist, Thambaram, Chennai.

6.2 IDENTIFICATION OF PHYTOCHEMICAL CONSTITUTENTS

6.2.1 PRELIMINARY PHYTOCHEMICAL TESTS :

The plant may be considered as a biosynthetic laboratory, not only for the chemical compounds such as Carbohydrates, Protein and Lipids that are utilized as food by men, but also for a multitude of compounds like Glycosides, Alkaloids, Volatile oils, Tannins etc., that exerts a physiologic effect. The compounds that are responsible for therapeutic effect are usually the secondary metabolites. A systemic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism. The plant material may be subjected to preliminary phytochemical screening for the detection of various plant constituents.

For our present study, we had taken the plant material as powdered plant of*Cayratiatrifolia*Gagnep. To extract the compounds are tested the chemical constituents present in them.

6.3 PREPARATION OF EXTRACTS

Preparation of the extracts of*Cayratiatrifolia*Gagneppowdered plant by using following solvents:

(a) Ethanol

(b) Distilled Water

(a) Ethanol extract

The shade dried course powder of the entire plant (250 gm) was packed well in soxhlet apparatus and was subjected for continuous hot extraction with 99.99% ethanol until the completion of the extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely. Dried and kept in a desiccator till experimentation. Obtained extract (dark blackish brown) was weighed and percentage yield was calculated in terms of air-dried powdered crude material (ethanolic extract was named as ETE).

(b) Aqueous extract

The shade dried course powder of the entire plant (250 gm) was packed well in soxhlet apparatus and was subjected to continuous hot extraction with distilled water until the completion of extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the distilled water completely. It was finally dried and kept in a desiccator till experimentation. Obtained extract (dark reddish brown) was weighed and percentage yield was calculated in terms of air-dried powdered crude material (ethanolic extract was named as AQE).

6.2 QUALITATIVE PHYTOCHEMICAL ANALYSIS

Both ethanolic and aqueous extracts obtained from the powdered plant*Cayratiatrifolia*gagnep .were subjected to various qualitative tests for the identification of various plant constituents present in this species.

1. Test for Alkaloids

(a) Dragendorff's Test: To 1 ml of the extract, add 1 ml of dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

(b) Mayer's Test: To 1 ml of the extract, add 1 ml of mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream colored precipitate indicates the presence of alkaloids.

(c) Hager's Test: To 1 ml of the extract, add 3ml of Hager's reagent (Saturated Petroleum Ether solution of picric acid), yellow colored precipitate indicates the presence of alkaloids.

(d) Wagner's Test : To 1 ml of the extract, add 2 ml of wagner's reagent (Iodine in Potassium Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

2. Test for Saponins

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins.

3. Test for Glycosides

(a) Legal Test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red color shows the presence of glycosides.

(b) Baljet Test: To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange color reveals the presence of glycosides.

(c) Keller-KillianiTest : 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelein dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

(d)Borntrager's Test : Add a few ml of dilute sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of

ammonia. The formation of red color of the ammonical layer shows the presence of anthraquinone glycosides.

4. Test for Carbohydrates and Sugars

(a) Molisch's Test: To 2ml of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

(b) Fehling's Test : To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars

(c) Benedict's test: To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

5. Test for Phenolic Compounds and Tannins

a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.
b) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.
c) The little quantity of test extract is treated with potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.
d) To the test extract, add strong potassium dichromate solution, a yellow colour precipitate indicates the presence of tannins and phenolics.

6. Test for Flavonoids

a) The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light, formation of fluorescence indicates the presence of flavonoids.
b) Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour solution formed, disappears on addition of an acid indicates the presence of flavonoids.
c) **Shinoda's Test:** The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red color indicates the presence of flavonones or orange red colour indicates the presence of flavonols.

d) The extract is treated with sodium hydroxide, formation of yellow colour indicates the presence of flavones.

e) The extract is treated with concentrated H_2SO_4, formation of yellow or orange colour indicates flavones.

f) The alcoholic and aqueous extract is treated with 10% sodium chloride; formation of yellow colour indicates the presence of coumarins.

7. Test for Steroids and Sterols

(a) Libermann-BurchardTest : 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.

(b) Salkowski Test: Dissolve the extract in chloroform and add equal volume of conc. H_2SO_4 .Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

8. Test for Proteins and Amino Acids

Biuret Test: Add 1ml of 40% sodium hydroxide solution and 2 drops of 1% $CuSO_4$ solution till a blue color is produced, then add to the 1ml of the extract. Formation of pinkish or purple violet colour indicates the presence of proteins.

Ninhydrin Test: Add two drops of freshly prepared 0.2% ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. Development of blue colour reveals the presence of proteins, peptides or amino acids.

Xanthoproteic Test: To 1ml of the extract, add 1ml of concentrated nitric acid. A white precipitate formed, it was boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. Orange colour indicates the presence of aromatic amino acids.

(a) Millon's Test: 1ml of test solution is made acidify with sulphuric acid and add millon's reagent and boil this solution. A yellow precipitate is formed indicates the presence of protein.

9.Test for Triterpenoids

(a) Noller's Test: Dissolve two or three granules or tin metal in 2ml thionyl chloride

solution. Then add 1ml of the extract into test tube and warm, the formation of pink colour indicates the presence of triterpenoids.

10. Test for Fixed Oils and Fats

(a) Spot Test: Press a small quantity of extracts between the filter paper. Oil stains on paper indicates the presence of fixed oils.

(b) Saponification test: To 1ml of the extract, add few drops of 0.5 N alcoholic Potassium hydroxide along with a drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

11. Test for Gums and Mucilage

(a) Add about 10ml of alcoholic extract slowly to 25ml of absolute alcohol with constant stirring. Filter the precipitate and dry in air. Examine the precipitate for its swelling properties and for the presence of carbohydrates.

12. Test for Lignins: With alcoholic solution of phloroglucinol and hydrochloric acid, the appearance of red colour shows the presence of lignins.

The constituents present in extracts of powdered plant of*Cayratiatrifoliagagnep* are given in table no - 6.

6.4 INVITRO ANTICANCER STUDIES

Among the various diseases attributed to mortality in humans all over the world, cancer is a leading cause. It was estimated that there were 10.9 million new cases, 6.7 million deaths, and 24.6 million persons living with cancer around the world (166). There is a necessity for search of new compounds with cytotoxic activity as the treatment of cancer with the available anticancer drugs is often unsatisfactory due to the problem cytotoxicity to the normal cells.

Plants have a long history of use in the treatment of cancer. Several studies have been conducted on herbs under a multitude of ethnobotanical grounds. Over the past few decades a significant progress has been made in cancer prevention and treatment. Plant-derived natural products are becoming important as anti-cancer derivatives, including vincristine, vinblastine,

paclitaxel and camptothecin, which are invaluable contributors of nature to modern medicine[38-41].

In recent years, *in vitro* toxicology has rapidly developed into a challenging new scientific discipline. Some of the complications occur during *in-vivo* cytotoxic screening that is intravenous administration of chemotherapeutic drugs cause significant individual differences in biotransformation and bio-distribution. To overcome this problem, *in-vitro* cytotoxic screenings are used in which the effect of chemotherapeutic drug is being studied in the tumor cells in culture outside the body. Also, *in vitro* toxicity tests have gained the support of many animal welfare organizations, and are seen as one of the most promising means whereby the reduction and replacement of animal usage can be achieved[42-44].

Principle:

The principle of this colorimetric assay is based on the capacity of mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, blue colored formazan product which is measured spectrophotometrically (174-175). Only viable cells with active mitochondria reduce significant amounts of MTT since the reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

Media:

Leibovitz L-15 Medium with L-Glutamine, FBS (Fetal Bovine Serum, SFM HEK-293 (Serum Free Media), Thioglycollate medium (TGM), Tryptone soya broth (TSB) and Cell proliferation kit (MTT) 1000 tests.

Cell lines:

MCF-7 (Breast cancer cell line) was purchased from NCCS, Pune.

Cell treatment procedure[45-48]:

Cytotoxicity of the plant extract on the MCF-7 breast cancer cell lines was determined using the MTT Proliferation assay kit. The cells in a concentration of 1×10 cells/ml were preincubated in culture medium for 3 hrs at 37 °C and 6.5 % CO_2. The cells were seeded at a concentration of 5×104 cells/well in 100 µl culture medium and at various concentrations (5 -100

µg/ml) of standard Methotrexate and extract (dissolved in 2 % DMSO (dimethyl sulphoxide) solution) into microplates (tissue culture grade, 96 wells, flat bottom) and incubated for 24 hrs at 37 °C and 6.5 % CO_2.

The test denotes the survival cells after toxic exposure. Then, 10 µl MTT labeling mixtures were added and incubated for 4 hrs at 37 °C and 6.5 % CO_2. Each experiment was done in triplicates. Then 100µl of solubilisation solution was added into each well and incubated for overnight. The spectrophotometric absorbance of the samples was measured using a microplate (ELISA) reader at a wavelength in between 550 and 600 nm according to the filters available for the ELISA reader. The reference wavelength should be more than 650 nm. Percentage inhibition of extract against all cell lines was calculated using the following formula:

% of cell survival = AT/AC x 100

AT– Absorbance of test

AC – absorbance of control

% of cell inhibition = 100 - % cell survival

The IC_{50} value, i.e., the concentration required to inhibit 50% of cell viability was determined by plotting the log of the drug concentration versus the percentage of inhibition. The best-fit line was plotted by least-squares linear regression. The 50% inhibitory concentration (IC_{50}) was calculated from the linear-regression equation: Log (CV_{50}) = m×log (IC_{50})+c; where m is the regression coefficient, c is the intercept of the line, log (IC_{50}) is the log of the 50% inhibitory concentration of the extract and log (CV_{50}) is the log value of 50% cell viability.

7. RESULTS AND DISSCUSSION

The plant *Cayratiatrifolia*Gagnepbelonging to family Vitaeceae was selected for the project. On the basis of ethanobotanical information, which reveals its uses against disease like wound, inflammation, fever, tumor etc. Literature survey showed that very less work has been performed on this plant.So we can validate scientifically for folk claim for its therapeutic activity. We have also undertakes its detailed, preliminary phytochemical and *invitro* pharmacological investigation to give an appropriate identification and rationalize its use as drug of therapeutic importance.

Preliminary phytochemical studies preformed by starting with purification of solvents. Then powdered whole plant *Cayratiatrifolia*Gagnepwere subjected for continuous hot extraction with ethanol and distilled water. The yield was found to be 10.24 %w/w for ethanolic extract (ETE) and 16.2%w/w for aqueous extract (AQE). These extracts were subjected to various qualitative phytochemical tests to identify the active constituents which showed presence of Alkaloids, Glycosides, Saponins, Carbohydrates, Tannin & Phenolic compounds, Triterpinoids, Steroids, Flavonoids, Fixed oil and fats. Ethanolic extract showed the presence of Alkaloids, Glycosides, Saponins, Carbohydrates, Tannins & Phenolic compounds Triterpinoids, Steroids, Flavonoids, Fixed oil and fats, Gum and mucilage and Lignins ,whereas the aqueous extract showed, presence of Glycosides, carbohydrates, Tannin, Phenolic compounds, Flavonoids and Lignins.

The yield and % yield of both ethanolic and aqueous extracts of powdered plant of*Cayratiatrifolia*gagnep were reported.

TABLE NO. 1 - EXTRACTION VALUES OF ETHNOLIC AND AQUEOUS EXTRACTS OF*CAYRATIA TRIFOLIA GAGNEP.*

S. No.	Extracts	Yield (gms.)	% Yield (w/w)
1.	Ethanol Extract (ETE)	25.60	10.24
2.	Aqueous Extract (AQE)	40.64	16.2

TABLE NO. -2

DATA FOR PRELIMINARY PHYTOCHEMICAL ANALYSIS OF ETHANOLIC AND AQUEOUS EXTRACTS OFEXTRACTS OF *CAYRATIA TRIFOLIA*GAGNEP.

Phytoconstituents	Ethanolic extract	Aqueous extract
Alkaloids	+	-
Saponins	+	-
Glycosides	+	+
Carbohydrates	+	+
Tannins,Phenolic compounds	+	+
Flavonoids	+	+
Steroids	+	+
Proteins and Amino acids	-	-
Triterpenoids	+	-
Fixed Oils and Fats	+	-
Gums and Mucilage	+	-
Lignins	+	+

***In vitro* cytotoxic studies against MCF-7 breast cancer cell line by MTT assay**

Cytotoxicity potential of AQE and ETE, extracts were determined using MTT assay against MCF-7 breast cancer cell line. A significant increase in the % of cytotoxic value of the AQE andETEtreated cells were noted when compared to the standard. The IC_{50} for cytotoxicity was found to be the standard was 29.68µg/ml and cells treated with ETE were 37.51 µg/ml being the most potent inhibitor. AQE treated cells indicated an IC_{50} value of 40.6 µg/ml. The highest percentage inhibition was found to 97%, 92% and 86% for Standard drug Methotrexate,

Aqueous Extract and Ethanolic Extract respectively. However, the percentage inhibition of cytotoxicity was found to be lower for both the extracts AQE and ETE when compared to the standard drug methotrexate. In addition, ETE showed better inhibition than the AQE. The R^2value for the standard, AQE & ETE were 0.9755, 0.9560 & 0.9485 respectively.

ABSORPTION DATA OF STD, AQE & ETETABLE :3

Absorbance at 570 nm STD	Absorbance at 570 nm AQE	Absorbance at 570 nm ETE
0.2469	0.0041	00042
0.1646	0.0457	0.0042
0.1881	0.0582	0.0295
0.2195	0.0915	0.0885
0.2508	0.1373	0.1349
0.2900	0.1831	0.1812
0.2979	0.1997	0.1897
0.2979	0.2330	0.2065
0.3136	0.2663	0.2571
0.3175	0.2788	0.2571
0.3253	0.2996	0.2866
0.3371	0.3246	0.2951
0.3371	0.3287	0.3246
0.3488	0.3454	0.3330
0.3528	0.3454	0.3372
0.3528	0.3579	0.3372
0.3567	0.3704	0.3499
0..3645	0.3787	0.3499
0.3645	0.3787	0.3583
0.3920	0.4162	0.4216

TABLE. 4

CYTOTOXIC ACTIVITY OF STANDARD AND EXTRACTS AGAINST MCF-7 BREAST CANCER CELL

Con. (µg/ml)	Log.Con	%inhibition of STD	%inhibition of AQE	%inhibition of ETE
5	0.69897	37	2	2
10	1	43	12	2
15	1.17609	49	15	8
20	1.30103	57	23	22
25	1.39794	65	34	33
30	1.47712	75	45	44
35	1.54407	77	49	46
40	1.60206	77	57	50
45	1.65321	81	65	62
50	1.69897	82	68	62
55	1.74036	84	73	69
60	1.77815	87	79	71
65	1.81291	87	80	78
70	1.8451	90	84	80
75	1.87506	91	84	81
80	1.90309	91	87	81
85	1.92941	92	90	84
90	1.95424	94	92	84
95	1.97972	94	92	86
100	2	97	92	86

Cytotoxic activity of standard and extracts against MCF-7 breast cancer cell line

***Invitro* cytotoxic studies on STD, AQE & EET (Fig. 18)**

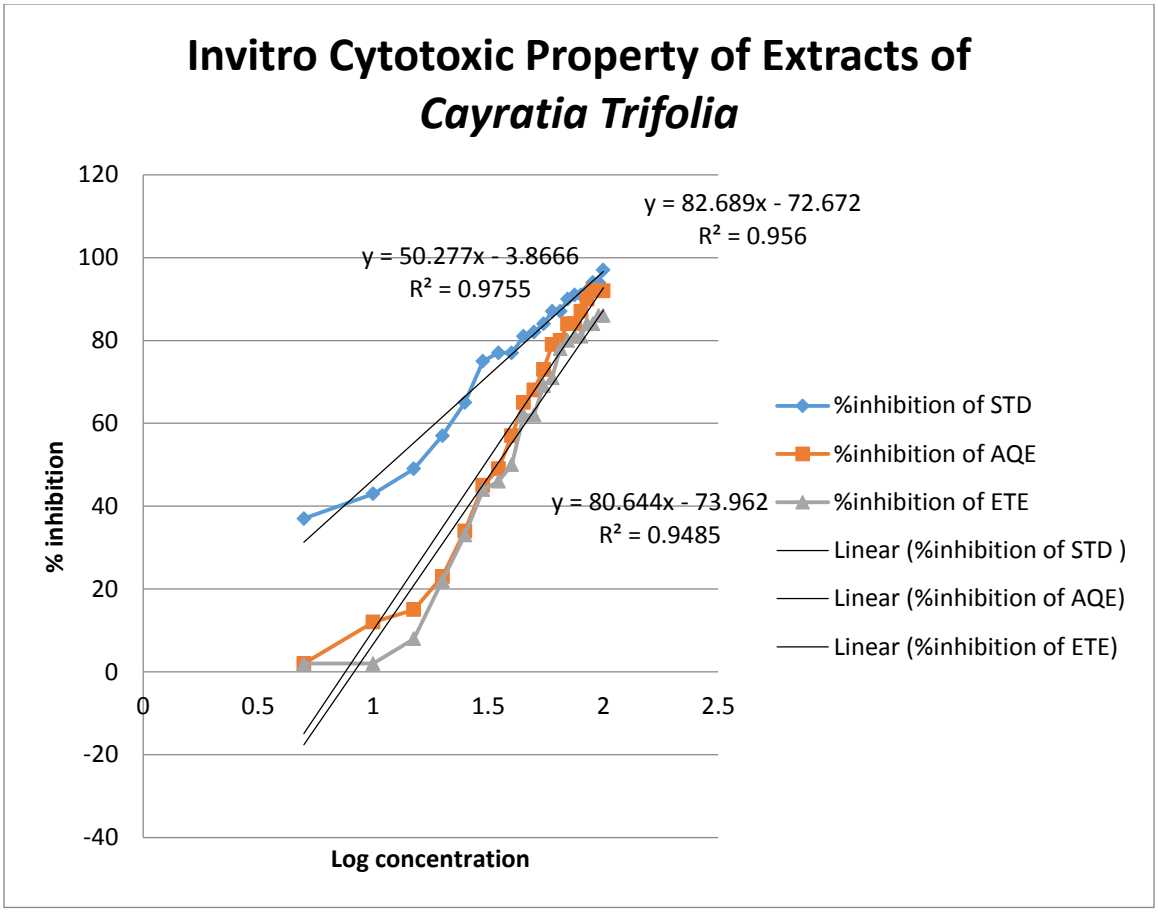


TABLE.5

LINEAR EQUATION, R^2 AND IC50 VALUES OF STD, AQE & ETE

S.No.	Sample	Linear equation	R^2	IC_{50} value
1	STD (Methotrexate)	Y = 50.277*x- 3.866	0.9755	29.68µg/ml
2	AQE	Y = 82.689*x-72.67	0.9560	40.6 µg/ml
3	ETE	Y = 80.644*x-73.96	0.9485	37.51µg/ml

The compounds of Tannins & Phenolic compounds Triterpinoids, Steroids and Flavonoids have been reported in ETE. The literatures proved that these compounds are potent antioxidants and free radical scavengers. The antioxidant, antimicrobial, and antitumor activities due to its phenolic, flavanoid and aromatic compounds. These beneficial substances can act as antioxidants and electrophile scavengers, stimulate the immune system, form the DNA addicts with carcinogens and induce detoxification enzymes. Hence, the reported cytotoxic activity of ETE may be due to the presence of polyphenolic compounds and antioxidant potential of the extracts.

8. CONCLUSIONS

The ethanolic and aqueous extracts of *Cayratiatrifolia*Gagnepbelonging to family Vitaeceaewere found to possess moderate cytotoxic potential with reference to the standard drug Methotrexate against MCF-7 breast cancer cell line. Among the extracts, the ethanolic extract showed better activity than the aqueous extract when comparing with standard.

The reported cytotoxic activity of the plant extracts in the present study may be due to the presence of phenolic and flavonoid constituents. This indicates the possibility of the plant extracts investigated for further development to cancer therapeutic agent and warrants further studies to understand the mechanisms of cytotoxic activity of the plant extract *Cayratiatrifolia*Gagnep.

However, the isolation of active principle will be advantageous to produce novel bioactive constituent from this extract which may possess more significant activity.

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CERTIFICATES

ACKNOWLEDGEMENT

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